MODULATION OF FLOWERING TIME BY THE PFT1 LOCUS

Government Support

[0001] This invention was made with United States government support under Grant No. GM52413 from the National Institutes of Health. The United States Government has certain rights in this invention.

Related Applications

[0002] This application claims priority to U.S. Patent Application No. 60/478,684, filed June 13, 2003, which is herein incorporated by reference in its entirety.

Background of the Invention

Field of the Invention

[0003] The field of the invention relates to methods of modulating at least one trait in a plant. Such traits include increased or decreased time to flowering and other traits associated with the shade avoidance syndrome. Also encompassed are transgenic plants produced by the disclosed methods.

Description of the Related Art

[0004] The influence of light on plant growth and development has been a topic of interest from the earliest studies of plant physiology. Flowering plants are subject to photoperiodism which is generally defined as the response of plants and animals to relative lengths of day and night. Plants are also sensitively attuned to differences in light quality. Red light, Far red light and blue light receptors are well characterized across plant species. One aspect of plant physiology that is particularly affected by photoperiodism and light quality is flowering.

[0005] The transition to flowering in plants is regulated by environmental factors such as temperature and light. In *Arabidopsis thaliana*, much is known about the photoperiod pathway that induces flowering in response to an increase in daylength. In contrast, the mechanisms that regulate flowering in response to changes in light quality

are largely unknown. In crowded or shaded environments, the red/far-red ratio of incoming light reaching plants decreases, and a series of responses known collectively as the "shade avoidance syndrome" are triggered, including the promotion of stem elongation and acceleration of flowering (Ballare, C.L. *Trends Plant Sci* 4, 201, 1999; and Halliday, K.J., et al. *Plant Physiol* 104, 1311-1315, 1994). Phytochromes are a family of red/far-red-light photoreceptors essential for the perception of changes in light quality and shade avoidance responses; among the 5 phytochromes in *Arabidopsis*, a phytochrome B (*phy*B) plays the most significant role in the shade avoidance syndrome. The mechanisms by which *phyB* regulates flowering are largely unknown.

[0006] The phytochromes and the blue/UV-A photoreceptors called cryptochromes (cry1 and cry2 in *Arabidopsis)* are the most critical photoreceptors that regulate floral induction (Lin, C. *Plant Physiol* 123, 39-50, 2000). Several components involved in phytochrome signaling in seedlings have been isolated and characterized in recent years (Quail, P.H. *Nat Rev Mol Cell Biol* 3, 85-93, 2002). Seedlings defective in phytochrome A (*phyA*) signaling are tall under far-red light (FR) while plants defective in *phyB* signaling are tall under red-light (R). Despite the large number of components identified, it remains unclear how they are assembled into a signaling network. *ELF3* and GI have been reported to have a role in flowering (Liu, X.L., et al. *Plant Cell* 13, 1293-304, 2001), mainly through mis-regulation of the circadian clock (Suarez-Lopez, P., et al. *Nature* 410, 116-20, 2001), but the mechanisms by which phytochromes regulate flowering directly are largely unknown. Using a new genetic screen for seedlings showing an enhanced response to R light and flowering time defects, a new recessive mutant, *pft1* (*phytochrome and flowering time 1*) was isolated; the *PFT1 locus* was cloned and characterized in detail. *PFT1* (PHYTOCHROME AND FLOWERING TIME 1) is a nuclear protein that plays an essential role in regulation of flowering time by *phyB*, acting downstream the photoreceptor to regulate the expression of the floral induction gene, *FLOWERING LOCUS T (FT)*. As used herein, upper case refers to the wild type form of *PFT1* and lower case refers to the mutant form.

Summary of the Invention

[0007 In one embodiment, the present invention includes a phytochrome and flowering time 1 (*PFT1*) protein or a nucleic acid molecule encoding a *PFT1* protein. In a preferred embodiment, the *PFT1* protein has the amino acid sequence set forth in SEQ ID

NO: 3 or SEQ ID NO: 17 or a conservative variant of the sequence shown in SEQ ID NO: 3 or SEQ ID NO: 17. In another preferred embodiment, the nucleic acid molecule has the sequence shown in SEQ ID NO: 1 or SEQ ID NO:2 or SEQ ID NO: 16 or homologous sequence to the sequence shown in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO: 16. In certain embodiments, the *PFT1* protein is at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 97%, at least about 50%, or at least about 99% identical to the sequence shown in SEQ ID NO: 3 or SEQ ID NO: 17. In other embodiments, the *PFT1* protein is encoded by a nucleotide sequence that hybridizes to SEQ ID NO:2 or SEQ ID NO: 16 under very high stringency hybridization, under high stringency hybridization, under moderate stringency hybridization or under low stringency hybridization. In still other embodiments, the *PFT1* protein encoding nucleic acid is at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 97%, at least about 50%, or at least about 99% identical to the sequence shown in SEQ ID NO: 1 or SEQ ID NO:2 or SEQ ID NO: 16.

[0008] In another embodiment, the present invention includes the above nucleic acids molecules operably linked to a promoter. In certain embodiments, the promoter may be a constitutive promoter, an inducible promoter, or regulated promoter such as a developmentally regulated, spatially regulated or temporally regulated promoter. In other embodiments, the promoter is functional in plants, in monocots, or in dicots. Another embodiment of the present invention includes any of the above nucleic acids in a vector or other genetic construct such as a viral genome.

[0009] In still another embodiment, the present invention includes transgenic plants expressing a *PFT1* protein as exemplified above or comprising any of the above nucleic acids, vectors or other constructs. In certain embodiments, the expression of the *PFT1* protein may be limited to particular times, or particular tissues, such as during the day or during the night, or in the seeds, fruits, fruiting bodies, leaves, stems, flowers, or roots. The present invention also includes transgenic plant parts such as seeds, fruits, fruiting bodies, leaves, stems, flowers, or roots. In certain embodiments, the plants may be wheat, barley, rye, oat, flax, millet, corn, tomato, rice and tobacco plants.

[0010] In one embodiment, the present invention is drawn to a method of modulating at least one photosensitive trait in a plant which includes altering the level of

phytochrome and flowering time 1 (*PFT1*) protein in a plant. In a preferred embodiment, the photosensitive trait is flowering time, shade avoidance syndrome, stem elongation or leaf number. In a preferred embodiment, the *PFT1* protein has the amino acid sequence set forth in SEQ ID NO. 3 or SEQ ID NO: 17or a conservative variant of the sequence shown in SEQ ID NO: 3 or SEQ ID NO: 17. In certain embodiments, the *PFT1* protein is at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 97%, at least about 50%, or at least about 99% identical to the sequence shown in SEQ ID NO: 3 or SEQ ID NO: 17. In other embodiments, the *PFT1* protein is encoded by a nucleotide sequence that hybridizes to SEQ ID NO:2 or SEQ ID NO: 16 under very high stringency hybridization, under high stringency hybridization, under moderate stringency hybridization or under low stringency hybridization.

[0011] In one embodiment, the level of *PFT1* protein is altered by producing a plant having an expression vector having a gene encoding the *PFT1* protein. In a preferred embodiment, the gene encoding the *PFT1* protein has a nucleotide sequence that encodes the amino acid sequence set forth in SEQ ID NO. 3 or SEQ ID NO: 17 or a conservative variant of the sequence shown in SEQ ID NO: 3 or SEQ ID NO: 17. In another preferred embodiment, the gene encoding the *PFT1* protein has the nucleotide sequence set forth in SEQ ID NO. 2 or SEQ ID NO: 16.

[0012] In one embodiment, the present invention is drawn to a method of modulating a photosensitive trait in a plant, including:

transforming a plant cell with an expression vector including a gene that encodes a *PFT1* protein; and

growing the plant cell into a plant under conditions that allow the expression of the *PFT1* protein thereby modulating a photosensitive trait.

[0013] In a preferred embodiment, the *PFT1* protein is overexpressed in the plant. In a preferred embodiment, the *PFT1* protein is encoded by a gene including the nucleotide sequence shown in SEQ ID NO: 2 or SEQ ID NO: 16. In another preferred embodiment, the *PFT1* protein is encoded by a gene including the nucleotide sequence shown in SEQ ID NO: 2, from nucleotides 1 to 2512. In one preferred embodiment, the expression vector includes a constitutive promoter. In an alternate preferred embodiment, the expression vector includes an inducible promoter.

[0014] Preferably, the plant is a tomato, rice or tobacco plant. In some preferred embodiments, the plant is *Arabidopsis thaliana*.

[0015] In a preferred embodiment, the photosensitive trait is a trait selected from the group including: flowering time, leaf number, stem elongation, and red/far red response. In a more preferred embodiment, the photosensitive trait is flowering time, and the flowering time is decreased.

[0016] In one embodiment, the present invention is drawn to a method of modulating a photosensitive trait in a plant which includes contacting a plant cell, or plant, with an inhibitor of a *PFT1* gene such that expression of the *PFT1* gene is reduced compared to a plant not contacted with the inhibitor. Preferably, the *PFT1* gene includes the nucleotide sequence shown in SEQ ID NO: 2 or SEQ ID NO: 16. In another preferred embodiment, the *PFT1* gene includes the nucleotide sequence shown in SEQ ID NO: 2, from nucleotide 1 to 2512.

[0017] In a preferred embodiment, the inhibitor includes an expression vector expressing a protein that inhibits expression of the *PFT1* gene. In a preferred embodiment, the plant is selected from the group including wheat, barley, rye, oat, flax, millet, corn, tomato, rice and tobacco plants.

[0018] In a preferred embodiment, the inhibitor includes an antisense molecule that inhibits the *PFT1* gene. In an alternate preferred embodiment, the inhibitor includes a short interfering RNA (siRNA) configured to inhibit the production of a *PFT1* gene product.

[0019] In a preferred embodiment, the photosensitive trait is a trait selected from the group including flowering time, leaf number, stem elongation, shade avoidance syndrome and Red/Far Red Response. In a more preferred embodiment, the photosensitive trait is flowering time, and said flowering time is increased. In a more preferred embodiment, the photosensitive trait is shade avoidance syndrome, and the plant exhibits a depressed shade avoidance syndrome.

[0020] In one aspect, the present invention is drawn to a transgenic plant having at least one modulated photosensitive trait as compared to a wild-type plant, wherein the transgenic plant includes a recombinant expression vector that expresses a nucleic acid encoding a *PFT1* gene. In a preferred embodiment, the *PFT1* gene is overexpressed. In a preferred embodiment, the *PFT1* gene includes the nucleotide sequence shown in SEQ ID NO: 2 or SEQ ID NO: 16. In another preferred embodiment,

the *PFT1* gene comprises the nucleotide sequence shown in SEQ ID NO: 2, from nucleotides 1 to 2512. In a preferred embodiment, the expression vector includes a constitutive promoter. In an alternate preferred embodiment, the expression vector includes an inducible promoter.

[0021] In a preferred embodiment, the transgenic plant is selected from the group including tomato, rice and tobacco plants. In alternate preferred embodiments, transgenic plant is *Arabidopsis thaliana*.

[0022] In a preferred embodiment, the photosensitive trait in the transgenic plant is a trait selected from the group including: flowering time, leaf number, stem elongation, and red/far red response. In a more preferred embodiment, the photosensitive trait is flowering time, and said flowering time is decreased.

[0023] In one aspect, the present invention is drawn to a seed derived from the transgenic plant described above. In a further aspect, the invention is drawn to a plant tissue derived from the transgenic plant described above. Preferably, the plant tissue is a flower.

[0024] In one aspect, the present invention is drawn to a transgenic plant having at least one modulated photosensitive trait as compared to a wild-type plant, wherein the transgenic plant includes a recombinant expression vector that produces an inhibitor of a *PFT1* gene. In a preferred embodiment, the *PFT1* gene includes the nucleotide sequence shown in SEQ ID NO: 2 or SEQ ID NO: 16. In an alternate preferred embodiment, the *PFT1* gene includes the nucleotide sequence shown in SEQ ID NO: 2, from nucleotides 1 to 2512. In a preferred embodiment, the expression vector includes a constitutive promoter. In an alternate preferred embodiment, the expression vector includes an inducible promoter.

[0025] In a preferred embodiment, the transgenic plant is a plant which is selected from the group including tomato, rice and tobacco plants. In an alternate preferred embodiment, the transgenic plant is *Arabidopsis thaliana*.

[0026] In a preferred embodiment, the inhibitor includes an antisense molecule that inhibits the *PFT1* gene. In an alternate preferred embodiment, the inhibitor includes a short interfering RNA (siRNA) configured to inhibit the production of a *PFT1* gene product.

[0027] In a preferred embodiment, the photosensitive trait is a trait selected from the group including flowering time, leaf number, shade avoidance syndrome, stem

elongation, and red/far red response. In a more preferred embodiment, the photosensitive trait is flowering time, and the flowering time is increased.

[0028] In one aspect, the present invention is drawn to a seed derived from the transgenic plant described above. In another aspect, the present invention is drawn to a plant tissue derived from the transgenic plant described above. In a preferred embodiment, the tissue is a flower.

[0029] In another aspect, the present invention includes methods of generating recombinant nucleic acid molecules encoding a *PTF1* protein as well as the recombinant nucleic acid molecules produced from such methods. The method includes providing genetic material from a plant and isolating from said nuclear material the nucleic acid molecule encoding a *PFT1* protein. In various embodiments, the genetic material may be genomic DNA, RNA, cDNA generated from a plant. In certain embodiments, the genetic material is encompassed in a library, which in certain embodiments may be an expression library. In certain embodiments, the plant may be selected from the group including wheat, barley, rye, oat, flax, millet, corn, tomato, rice and tobacco plants. The nucleic acid molecule may be isolated by any method available to one of ordinary skill in the art. In certain embodiments, the nucleic acid molecule is isolated by hybridization to a *PFT1* encoding polynucleotide or fragment thereof. Examples of such isolation include hybridization to amplify the nucleic acid molecule, hybridization to identify the nucleic acid molecule in a library, and hybridization to directly purify the nucleic acid molecule. In another embodiment, the isolation is performed by screening an expression library with an antibody to a *PFT1* protein including without limitation the *PFT1* proteins disclosed herein.

Brief Description of the Drawings

[0030] **Figure 1A-B. Genomic sequence of *PFT1*.** The *PFT1* genomic sequence is shown with upstream and downstream sequences. The numbering is according to BAC F2J7. The sequence corresponds to SEQ ID NO: 1. Exons are underlined. Intron donor and acceptor sites are in bold, italic. Transcription initiation is shown in caps, italic. Start and stop codons are in caps, bold.

[0031] **Figure 2. cDNA sequence of *PFT1*.** This sequence corresponds to SEQ ID NO: 2. Start and stop codons are in caps, bold and underlined.

[0032] **Figure 3. Protein sequence of *PFT1*.** This sequence corresponds to SEQ ID NO: 3.

[0033] **Figure 4. Phenotypes of *pft1*, *phyA* and *phyB* single, double and triple mutants.** **a**, Hypocotyl length of 5-day-old seedlings of the indicated genotypes grown for 4 days under 3 μmol/m^{2}s of far-red-light (FR) or 10 μmol/m^{2}s of red-light (R). **b,c** Flowering time of the indicated genotypes grown under long days (16 h WL/8h D) (**b**) and short days (9 h WL/16 h D) (**c**). Data represent averages of 3 independent experiments (**a**) and 8-10 plants (**b-c**) ± SE.

[0034] **Figure 5. Molecular characterization of *PFT1*.** **a**, structure of the *PFT1* gene, black boxes represent exons and lines introns. Predicted domains and the T-DNA insertion (out of scale) are indicated. Scale bar = 1 kb. **b, c, d,** Molecular complementation of the hypocotyl length phenotype (**b**), late flowering phenotype in long days (**c**) and delayed bolting in short days (**d**). Different transgenic lines for the *PFT1* gene were used, as indicated. OX1 and OX2 are 35S-*PFT1* overexpressor lines in *pft1* and WT backgrounds, respectively, COS a cosuppressed line in *pft1* background and G1 a complemented line.

[0035] **Figure 6. *FT* and *CO* mRNA levels in WT, *phyB*, *pft1* and *pft1 phyB* mutants.** **a**, quantification of *FT* and *CO* expression in 8 day-old seedlings grown under long-day and harvested at the indicated time (0 = lights on) relative to the *UBQ10* control. **b**, quantification of *FT* and *CO* expression in 26 day-old seedlings grown under long-day and harvested at the indicated time (0 = lights on) relative to the *UBQ10* control. Data in **a** and **b** are representative of two independent experiments.

[0036] **Figure 7. *FT* and *CO* overexpression suppress the *pft1* flowering time phenotype.** **a**, flowering time of WT, *pft1*, *FT* overexpressor in WT (*FT OX*) and *pft1* (*pft1 FT OX*) backgrounds. **b**, flowering time of WT (cv Landsberg *erecta*), *pft1*, *CO* overexpressor in WT (*CO OX*) and *pft1* (*pft1 CO OX*) backgrounds. For this particular experiment the *pft1* mutation was introgressed 4 times into the *Landsberg erecta* ecotype before crossing to *CO OX* lines. Data from (**a**) and (**b**) are media ± SE of at least 10 plants. **c**, A proposed model to explain *PFT1* involvement in flowering time. With the exception of the direct regulation of *FT* by *CO* (Samach, A. et al. *Science* 288, 1613-6, 2000), other arrows do not represent direct regulation or interaction. *phyA* and cry2 appear to mediate a direct effect of light on *CO* activity (Yanovsky, M.J., et al. *Nature* 419, 308-12, 2002). The role of *FT* in the proposed pathway is based on previous studies (Kardailsky, I., et al. *Science* 286, 1962-5, 1999; and Kobayashi, Y., et al. *Science* 286, 1960-2, 1999). The placement of *PFT1* is deduced from this work.

[0037] **Figure 8. *PFT1* expression in WT and *phyB* plants.** **a**, *pft1* and 25S expression in WT and *phyB* plants under SD and LD conditions. Seedlings were harvested at the indicated times (0= lights on). **b**, quantification of data shown in (a) relative to the 25S control. **c**, *PFT1* and 25S expression in WT and *phyB* plants. Seedlings were grown under (LD or SD) conditions and harvested at the times indicated (0= lights on). **d**, quantification of data shown in (c) relative to the 25S control.

[0038] **Figure 9A-C. Alignment of *PFT1* genes.** The alignment of *PFT1* genes from five plants using ClustalW (Ver. 1.82) is shown. The genes include rice (OsPFT1), sacharum officinarum (SoPFT1), medicago (MtPFT1), sorghum (SbPFT1) and Arabidopsis (AtPFT1). **Part 1.** Alignment scores (% identity) from pairwise comparison of the five sequences and **Part 2.** alignment of the five sequences.

[0039] **Figure 10. Genomic sequence of rice *PFT1*.** The rice *PFT1* genomic sequence is shown with upstream and downstream sequences. The numbering is according to BAC OSJNBa0064I23. The sequence corresponds to SEQ ID NO: 16. Exons are underlined. Intron donor and acceptor sites are in bold, italic. Transcription initiation is shown in caps, italic. Start and stop codons are in caps, bold.

[0040] **Figure 11. Protein sequence of rice *PFT1*.** This sequence corresponds to SEQ ID NO: 17.

Detailed Description of the Preferred Embodiment

[0041] Embodiments of the invention are based, in part, upon the identification of an EST clone (APZL03h11R) as a novel gene regulating flowering time in plants corresponding to the *PFT1* locus. Thus, one embodiment of the invention provides isolated nucleic acids including nucleotide sequences comprising or derived from the *PFT1* genes and/or encoding polypeptides comprising or derived from the *PFT1* proteins. *PFT1* sequences include the specifically disclosed sequence, and splice variants, allelic variants, synonymous sequences, and homologous or orthologous variants thereof. Thus, for example, embodiments of the invention include genomic and cDNA sequences from the *PFT1* gene.

[0042] Embodiments of the invention also include allelic variants and homologous or orthologous sequences. For example, these variants are useful in allele specific hybridization screening or PCR amplification techniques. Moreover, subsets of the *PFT1* sequence, including both sense and antisense sequences, and both normal and

mutant sequences, as well as intronic, exonic and untranslated sequences, may be employed for these techniques. Such sequences may comprise a small number of consecutive nucleotides from the sequence disclosed or otherwise enabled herein but preferably include at least 8-10, and more preferably 9-25, consecutive nucleotides from an *PFT1* sequence. Various nucleic acid constructs in which *PFT1* sequences, either complete or subsets, are operably joined to exogenous sequences to form cloning vectors, expression vectors, fusion vectors, transgenic constructs, and the like are also contemplated.

[0043] The *PFT1* mutant was identified in a screening assay for T-DNA activation tagged *Arabidopsis* lines. Accordingly, embodiments of the invention include the *PFT1* gene and mutations thereof, as well as a characterization of the *PFT1* mutant which has been discovered in *Arabidopsis*. However, the disclosed methods are not limited to any particular plant type. It is expected that similar mutations in other plants will result in similar phenotypes.

[0044] Embodiments of the invention also include functional *PFT1* polypeptides, and functional fragments thereof. As used herein, the term "functional polypeptide" refers to a polypeptide which possesses biological function or activity which is identified through a defined functional assay and which is associated with a particular biologic, morphologic, or phenotypic alteration in the cell. The term "functional fragments of *PFT1* polypeptide", refers to all fragments of *PFT1* that retain *PFT1* activity, e.g., ability to confer a modulated photosensitive trait such as an altered flowering time and/or leaf number in a plant. Biologically functional fragments, for example, can vary in size from a polypeptide fragment as small as an epitope capable of binding an antibody molecule to a large polypeptide capable of participating in the characteristic induction or programming of phenotypic changes within a cell. "Photosensitive trait" refers to a plant trait that is mediated through one or more light receptors including but not limited to a phytochrome photoreceptor, a cryptochrome photoreceptor, a ztl receptor, and a phototroping receptor.

[0045] Many modifications of the *PFT1* primary amino acid sequence may result in plants having reduced or abolished *PFT1* responses. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the polypeptides produced by these modifications are included herein as long as the biological activity of *PFT1* is present. Further, deletion of one or more amino acids can also result

in a modification of the structure of the resultant molecule without significantly altering its activity. This can lead to the development of a smaller active molecule which could have broader utility. For example, it may be possible to remove amino or carboxy terminal amino acids required for *PFT1* activity.

[0046] *PFT1* polypeptides include amino acid sequences substantially the same as the sequence set forth in SEQ ID NO: 3 or SEQ ID NO: 17, including mutants that result in plants having altered time to flowering and/or leaf number. The term "substantially the same" refers to amino acid sequences that provide nearly the same amino acid sequence, or retain the activity of *PFT1* as described herein. The *PFT1* polypeptides of the invention include conservative variations of the polypeptide sequence.

[0047] The term "conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide.

[0048] Figure 9 shows the amino acid sequence alignment of several *PF1* proteins. While a number of the amino acid sequences are only partial sequences from various databases, the sequence alignment shows which regions of the protein are more conserved that the others. In addition, one of skill in the art may perform additional sequence alignments using other known methods. Such sequence alignments provide a good indication of the degree of variation of amino acid residues at any given position that may be tolerated. One of skill in the art would understand that highly conserved regions are less likely to tolerate significant variation while less conserved regions are more likely to tolerate variation. Also, one of skill in the art will appreciate that where corresponding residues vary between the sequences, such variation gives an indication of the nature of changes that are likely to be tolerated without disturbing the function of the protein. However, one of skill in the art also will appreciate that some areas such as the C-terminus, while less conserved on an absolute level, have significant motifs that are likely to be functional, such as the glutamine rich region.

[0049] *PFT1* proteins can be analyzed by standard SDS-PAGE and/or immunoprecipitation analysis and/or Western blot analysis, for example. Embodiments of the invention also provide an isolated polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 3 or SEQ ID NO: 17. The term "isolated" as used herein includes polynucleotides substantially free of other nucleic acids, proteins, lipids, carbohydrates or other materials with which it is naturally associated. Polynucleotide sequences of the invention include DNA, cDNA and RNA sequences which encode *PFT1*. It is understood that polynucleotides encoding all or varying portions of *PFT1* are included herein, as long as they encode a polypeptide with *PFT1* activity. Such polynucleotides include naturally occurring, synthetic, and intentionally manipulated polynucleotides as well as splice variants. For example, portions of the mRNA sequence may be altered due to alternate RNA splicing patterns or the use of alternate promoters for RNA transcription.

[0050] Moreover, *PFT1* polynucleotides include polynucleotides having alterations in the nucleic acid sequence which still encode a polypeptide having the ability to modulate a photosensitive trait such as the flowering response and/or leaf number. Alterations in *PFT1* nucleic acid include but are not limited to intragenic mutations (e.g., point mutation, nonsense (stop), antisense, splice site and frameshift) and heterozygous or homozygous deletions. Detection of such alterations can be done by standard methods known to those of skill in the art including sequence analysis, Southern blot analysis, PCR based analyses (e.g., multiplex PCR, sequence tagged sites (STSs)) and *in situ* hybridization. Embodiments of the invention also include anti-sense polynucleotide sequences.

[0051] The polynucleotides described herein include sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the invention as long as the amino acid sequence of *PFT1* polypeptide encoded by such nucleotide sequences retains *PFT1* activity. A "functional polynucleotide" denotes a polynucleotide which encodes a functional polypeptide as described herein. In addition, embodiments of the invention also include a polynucleotide encoding a polypeptide having the biological activity of an amino acid sequence of SEQ ID NO: 3 or SEQ ID NO: 17 and having at least one epitope for an antibody immunoreactive with *PFT1* polypeptide.

[0052] As used herein, the terms "polynucleotides" and "nucleic acid sequences" refer to DNA, RNA and cDNA sequences.

[0053] The polynucleotides encoding *PFT1* include the nucleotide sequence of SEQ ID NOS: 1, 2, and 16. Genomic DNA sequences are shown in SEQ ID NO: 1 and 16. A cDNA sequence is shown in SEQ ID NO: 2. Nucleic acid sequences complementary to SEQ ID NOS: 1, 2, and 16 are also encompassed within the present invention. A complementary sequence may include an antisense nucleotide. When the sequence is RNA, the deoxyribonucleotides A, G, C, and T of SEQ ID NOS: 1 and 2 and 16 are replaced by ribonucleotides A, G, C, and U, respectively. Also included in the invention are fragments ("probes") of the above-described nucleic acid sequences that are at least 15 bases in length, which is sufficient to permit the probe to selectively hybridize to DNA that encodes the protein of SEQ ID NO: 3 or SEQ ID NO: 17.

[0054] "Antisense" nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule (Weintraub, *Scientific American* 262 40, 1990). In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule. This interferes with the translation of the mRNA since the cell will not translate a mRNA that is double-stranded. Antisense oligomers of about 15 nucleotides are preferred, since they are easily synthesized and are less likely to cause non-specific interference with translation than larger molecules. The use of antisense methods to inhibit the in vitro translation of genes is well known in the art (Marcus-Sakura *Anal. Biochem.* 172: 289, 1998). In the present case, plants transformed with constructs containing antisense fragments of the *PFT1* gene would display a modulated photosensitive phenotype such as altered time to flowering.

[0055] Long double-stranded RNAs (dsRNAs; typically >200 nt) can be used to silence the expression of target genes in plants and plant cells. Upon introduction, the long dsRNAs enter the RNA interference (RNAi) pathway which involves the production of short (20-25 nucleotide) small interfering RNAs (siRNAs) and assembly of the siRNAs into RNA-induced silencing complexes (RISCs). The siRNA strands are then unwound to form activated RISCs. which cleave the target RNA. Double stranded RNA has been shown to be extremely effective in silencing a target RNA. Introduction of double stranded RNA corresponding to the *PFT*1 gene would be expected to modify the photosensitive phenotypes discussed herein including, but not limited to, flowering time, leaf number and stem length.

[0056] "Hybridization" refers to the process by which a nucleic acid strand joins with a complementary strand through base pairing. Hybridization reactions can be sensitive and selective so that a particular sequence of interest can be identified even in samples in which it is present at low concentrations. Suitably stringent conditions can be defined by, for example, the concentrations of salt or formamide in the prehybridization and hybridization solutions, or by the hybridization temperature, and are well known in the art. In particular, stringency can be increased by reducing the concentration of salt, increasing the concentration of formamide, or raising the hybridization temperature.

[0057] For example, hybridization under high stringency conditions could occur in about 50% formamide at about 37°C to 42°C. Hybridization could occur under reduced stringency conditions in about 35% to 25% formamide at about 30°C to 35°C. In particular, hybridization could occur under high stringency conditions at 42°C in 50% formamide, 5X SSPE, 0.3% SDS, and 200 ng/ml sheared and denatured salmon sperm DNA. Hybridization could occur under medium stringency conditions as described above, but in 35% formamide at a reduced temperature of 35°C. The temperature range corresponding to a particular level of stringency can be further narrowed by calculating the purine to pyrimidine ratio of the nucleic acid of interest and adjusting the temperature accordingly. Variations on the above ranges and conditions are well known in the art. "Selective hybridization" as used herein refers to hybridization under moderately stringent or highly stringent physiological conditions (See, for example, the techniques described in Maniatis et al., 1989 Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y., incorporated herein by reference), which distinguishes related from unrelated *PFT1* nucleotide sequences.

[0058] In another aspect of the invention, very high stringency hybridization conditions can include at least one wash at 0.1 x SSC, 0.1 % SDS, at 60°C for 15 minutes. High stringency hybridization conditions can include at least one wash at 0.2 x SSC, 0.1 % SDS, at 60°C for 15 minutes. Moderate stringency hybridization conditions can include at least one wash at 0.5 x SSC, 0.1 % SDS, at 60°C for 15 minutes. Low stringency hybridization conditions can include at least one wash at 1.0 x SSC, 0.1 % SDS, at 60°C for 15 minutes.

[0059] Another aspect of the invention is polypeptides or fragments thereof which have at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or more than about 95% homology to SEQ ID NO: 3 or SEQ ID

NO: 17, and sequences substantially identical thereto, or a fragment comprising at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids thereof. Homology may be determined using any of the methods described herein which align the polypeptides or fragments being compared and determines the extent of amino acid identity or similarity between them. It will be appreciated that amino acid "homology" includes conservative amino acid substitutions such as those described above.

[0060] The polypeptides or fragments having homology to SEQ ID NO: 3 or SEQ ID NO: 17, and sequences substantially identical thereto, or a fragment comprising at least about 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids thereof may be obtained by isolating the nucleic acids encoding them using the techniques described herein.

[0061] Alternatively, the homologous polypeptides or fragments may be obtained through biochemical enrichment or purification procedures. The sequence of potentially homologous polypeptides or fragments may be determined by proteolytic digestion, gel electrophoresis and/or microsequencing. The sequence of the prospective homologous polypeptide or fragment can be compared to the polypeptide of SEQ ID NO: 3 or SEQ ID NO: 17, and sequences substantially identical thereto, or a fragment comprising at least about 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids thereof using any of the programs described above.

[0062] Also included in embodiments of the invention are nucleotide sequences that are greater than 70% homologous with SEQ ID NOS: 1 or 2 or 16, but still retain the ability to modulate a photosensitive trait such as an altered time to flowering. Other embodiments of the invention include nucleotide sequences that are greater than 75%, 80%, 85%, 90% or 95% homologous with SEQ ID NOS: 1 or 2 or 16, but still retain the ability to confer a modulated photosensitive phenotype which includes altered time to flowering.

[0063] Specifically disclosed herein is a cDNA sequence for *PFT1*, as well as two genomic DNA sequences. DNA sequences of the invention can be obtained by several methods. For example, the DNA can be isolated using hybridization or computer-based techniques which are well known in the art. Such techniques include, but are not limited to: 1) hybridization of genomic or cDNA libraries with probes to detect homologous nucleotide sequences; 2) antibody screening of expression libraries to detect cloned DNA fragments with shared structural features; 3) polymerase chain reaction

(PCR) on genomic DNA or cDNA using primers capable of annealing to the DNA sequence of interest; 4) computer searches of sequence databases for similar sequences; and 5) differential screening of a subtracted DNA library.

[0064] Screening procedures which rely on nucleic acid hybridization make it possible to isolate any gene sequence from any organism, provided the appropriate probe is available. Oligonucleotide probes, which correspond to a part of the *PFT1* sequence encoding the protein in question, can be synthesized chemically. This requires that short, oligopeptide stretches of the amino acid sequence must be known. The DNA sequence encoding the protein can be deduced from the genetic code, however, the degeneracy of the code must be taken into account. It is possible to perform a mixed addition reaction when the sequence is degenerate. This includes a heterogeneous mixture of denatured double-stranded DNA. For such screening, hybridization is preferably performed on either single-stranded DNA or denatured double-stranded DNA. Hybridization is particularly useful in the detection of cDNA clones derived from sources where an extremely low amount of mRNA sequences relating to the polypeptide of interest are present. In other words, by using stringent hybridization conditions directed to avoid non-specific binding, it is possible, for example, to allow the autoradiographic visualization of a specific cDNA clone by the hybridization of the target DNA to that single probe in the mixture which is its complete complement (Wallace, et al., *Nucl. Acid Res.* 9, 879, 1981). Alternatively, a subtractive library is useful for elimination of non-specific cDNA clones.

[0065] Among the standard procedures for isolating cDNA sequences of interest is the formation of plasmid- or phage-carrying cDNA libraries which are derived from reverse transcription of mRNA which is abundant in donor cells that have a high level of genetic expression. When used in combination with polymerase chain reaction technology, even rare expression products can be cloned. In those cases where significant portions of the amino acid sequence of the polypeptide are known, the production of labeled single or double-stranded DNA or RNA probe sequences duplicating a sequence putatively present in the target cDNA may be employed in DNA/DNA hybridization procedures which are carried out on cloned copies of the cDNA which have been denatured into a single-stranded form (Jay, et al. *Nucl. Acid Res.*, 11, 2325, 1983).

[0066] A cDNA expression library, such as lambda gt11, can be screened indirectly for *PFT1* peptides using antibodies specific for *PFT1*. Such antibodies can be

either polyclonally or monoclonally derived and used to detect expression product indicative of the presence of *PFT1* cDNA.

[0067] Another embodiment of the invention relates to plants that have at least one modulated photosensitive trait. Such modifications might include an altered red / far red response which refers to the response of a plant to red and far red light. "Red / far red response" is mediated through phytochromes which exist in two interconvertible forms: P_R absorbs red (about 660 nm) light and P_{FR} which absorbs far red (about 730 nm) light. Thus, a red / far red response is a plant response mediated by a phytochrome. Phytochrome mediates a wide variety of responses including shade avoidance syndrome. "Shade avoidance syndrome" refers to a series of responses triggered in crowded or shaded environments by a decrease in the red/far-red ratio of incoming light reaching the plants and includes the promotion of stem elongation and acceleration of flowering.

[0068] Specific modifications include transgenic plants with an altered time to flowering or an altered leaf number due to transformation with constructs using antisense or siRNA technology that affect transcription or expression from the *PFT1* gene. Such plants exhibit an altered time to flowering and leaf number. "Flowering time" refers to the number of days to flower. Some plants germinate better if seeds are cold treated. The plants are then moved to growing conditions and this is taken as time 0 for determination of time to flowering. If there is no pre-treatment, then time 0 starts at planting. Both increases and decreases in time to flowering are encompassed herein.

[0069] Accordingly, in another series of embodiments, the present invention provides methods of screening or identifying proteins, small molecules or other compounds which are capable of inducing or inhibiting the expression of the *PFT1* genes and proteins. The assays may be performed in vitro using transformed or non-transformed cells, immortalized cell lines, or in vivo using transformed plant models enabled herein. In particular, the assays may detect the presence of increased or decreased expression of *PFT1* (from *Arabidopsis* or other plants) genes or proteins on the basis of increased or decreased mRNA expression, increased or decreased levels of *PFT1* protein products, or increased or decreased levels of expression of a marker gene (e.g., beta-galactosidase, green fluorescent protein, alkaline phosphatase or luciferase) operably joined to an *PFT1* 5' regulatory region in a recombinant construct. Cells known to express a particular *PFT1*, or transformed to express a particular *PFT1*, are incubated and one or more test compounds are added to the medium. After allowing a sufficient period

of time (e.g., 0-72 hours) for the compound to induce or inhibit the expression of *PFT1*, any change in levels of expression from an established baseline may be detected using any of the techniques described above.

[0070] In another series of embodiments, the present invention provides methods for identifying proteins and other compounds which bind to, or otherwise directly interact with, the *PFT1*. The proteins and compounds include endogenous cellular components which interact with *PFT1 in vivo* and which, therefore, provide new targets for agricultural products, as well as recombinant, synthetic and otherwise exogenous compounds which may have *PFT1* binding capacity and, therefore, are candidates for modulating photosensitive traits. Thus, in one series of embodiments, High Throughput Screening-derived proteins, DNA chip arrays, cell lysates or tissue homogenates may be screened for proteins or other compounds which bind to one of the normal or mutant *PFT1* genes. Alternatively, any of a variety of exogenous compounds, both naturally occurring and/or synthetic (e.g., libraries of small molecules or peptides), may be screened for *PFT1* binding capacity.

[0071] In each of these embodiments, an assay is conducted to detect binding between *PFT1* and another moiety. The *PFT1* in these assays may be any polypeptide comprising or derived from a normal or mutant *PFT1* protein, including functional domains or antigenic determinants of *PFT1*. Binding may be detected by non-specific measures (e.g., transcription modulation, altered chromatin structure, peptide production or changes in the expression of other downstream genes which can be monitored by differential display, 2D gel electrophoresis, differential hybridization, or SAGE methods) or by direct measures such as immunoprecipitation, the Biomolecular Interaction Assay (BIAcore) or alteration of protein gel electrophoresis. The preferred methods involve variations on the following techniques: (1) direct extraction by affinity chromatography; (2) co-isolation of *PFT1* components and bound proteins or other compounds by immunoprecipitation; (3) BIAcore analysis; and (4) the yeast two-hybrid systems.

[0072] Embodiments of the invention also include methods of identifying proteins, small molecules and other compounds capable of modulating the activity of normal or mutant *PFT1*. Using normal cells or plants, the transformed cells and plant models of the present invention, or cells obtained from subjects bearing normal or mutant *PFT1* genes, the present invention provides methods of identifying such compounds on the basis of their ability to affect the expression of *PFT1*, the activity of *PFT1*, the activity

of other *PFT1*-regulated genes, the activity of proteins that interact with normal or mutant *PFT1* proteins, the intracellular localization of the *PFT1*, changes in transcription activity, the presence or levels of membrane bound *PFT1*, or other biochemical, histological, or physiological markers which distinguish cells bearing normal and modulated *PFT1* activity in plants.

[0073] In accordance with another aspect of the invention, the proteins of the invention can be used as starting points for rational chemical design to provide ligands or other types of small chemical molecules. Alternatively, small molecules or other compounds identified by the above-described screening assays may serve as "lead compounds" in design of modulators of photosensitive traits in plants.

[0074] DNA sequences encoding *PFT1* can be expressed in vitro by DNA transfer into a suitable host cell. "Host cells" are cells in which a vector can be propagated and its DNA expressed. The term also includes any progeny or graft material, for example, of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used. Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art.

[0075] As part of the present invention, the *PFT1* polynucleotide sequences may be inserted into a recombinant expression vector. The terms "recombinant expression vector" or "expression vector" refer to a plasmid, virus or other vehicle known in the art that has been manipulated by insertion or incorporation of the *PFT1* genetic sequence. Such expression vectors contain a promoter sequence which facilitates the efficient transcription of the inserted *PFT1* sequence. The expression vector typically contains an origin of replication, a promoter, as well as specific genes which allow phenotypic selection of the transformed cells.

[0076] Methods which are well known to those skilled in the art can be used to construct expression vectors containing the *PFT1* coding sequence and appropriate transcriptional/translational control signals. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo recombination/genetic techniques.

[0077] A variety of host-expression vector systems may be utilized to express the *PFT1* coding sequence. These include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid

DNA expression vectors containing the *PFT1* coding sequence; yeast transformed with recombinant yeast expression vectors containing the *PFT1* coding sequence; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing the *PFT1* coding sequence; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the *PFT1* coding sequence; or animal cell systems infected with recombinant virus expression vectors (e.g., retroviruses, adenovirus, vaccinia virus) containing the *PFT1* coding sequence, or transformed animal cell systems engineered for stable expression.

[0078] Depending on the host/vector system utilized, any of a number of suitable transcription and translation elements, including constitutive and inducible promoters, transcription enhancer elements, transcription terminators, etc. may be used in the expression vector (see e.g., Bitter et al. *Methods in Enzymology* 153, 516-544, 1987). For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage γ, plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like may be used. When cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the retrovirus long terminal repeat; the adenovirus late promoter; the vaccinia virus 7.5K promoter) may be used. Promoters produced by recombinant DNA or synthetic techniques may also be used to provide for transcription of the inserted *PFT1* coding sequence.

[0079] Isolation and purification of recombinantly expressed polypeptide, or fragments thereof, may be carried out by conventional means including preparative chromatography and immunological separations involving monoclonal or polyclonal antibodies.

[0080] In another embodiment, embodiments of the invention provide a method for producing a genetically modified plant having at least one modulated photosensitive trait such as having an altered time to flowering as compared to a plant which has not been genetically modified (e.g., a wild-type plant). The method includes the steps of contacting a plant cell with at least one vector containing at least one nucleic acid sequence encoding an *PFT1* gene or a mutant, homolog or fragment thereof, wherein the nucleic acid sequence is operably associated with a promoter, to obtain a transformed plant cell; producing a plant from the transformed plant cell; and thereafter selecting a plant exhibiting a modulated photosensitive trait such as an altered time to flowering.

[0081] Transgenic plants that result in at least one modulated photosensitive trait such as an altered time to flowering may be obtained by reduced expression of the *PFT1* gene. Thus, one embodiment of the invention includes plants transformed with antisense polynucleotides complementary to the *PFT1* gene or fragments thereof wherein production of the antisense polynucleotides results in reduced expression of the *PFT1* gene. In an alternate embodiment, reduced expression of *PFT1* may also be achieved by methods such as cosuppression (Hooper, C. *J. NIH Res.* 3, 49-54, 1991) by operatively linking a truncated form of an *PFT1* gene to a promoter. In an alternate embodiment, transgenic plants overexpressing the *PFT1* gene are described. Such plants might be expected to display a modulated photosensitive trait such as an altered time to flowering.

[0082] The term "genetic modification" as used herein refers to the introduction of one or more heterologous nucleic acid sequences, e.g., an *PFT1* or an *PFT1* mutant encoding sequence, into one or more plant cells, which can generate whole, sexually competent, viable plants. The term "genetically modified" as used herein refers to a plant which has been generated through the aforementioned process. Genetically modified plants of the invention are capable of self-pollinating or cross-pollinating with other plants of the same species so that the foreign gene, carried in the germ line, can be inserted into or bred into agriculturally useful plant varieties. The term "plant cell" as used herein refers to protoplasts, gamete producing cells, and cells which regenerate into whole plants. Accordingly, a seed comprising multiple plant cells capable of regenerating into a whole plant, is included in the definition of "plant cell".

[0083] As used herein, the term "plant" refers to either a whole plant, a plant part, a plant cell, or a group of plant cells, such as plant tissue, for example. Plantlets are also included within the meaning of "plant". Plants included in the invention are any plants amenable to transformation techniques, including angiosperms, gymnosperms, monocotyledons and dicotyledons.

[0084] Examples of monocotyledonous plants include, but are not limited to, asparagus, field and sweet corn, barley, wheat, rice, sorghum, onion, pearl millet, rye and oats. Examples of dicotyledonous plants include, but are not limited to tomato, tobacco, cotton, potato, rapeseed, field beans, soybeans, peppers, lettuce, peas, alfalfa, clover, cole crops or *Brassica oleracea* (e.g., cabbage, broccoli, cauliflower, brussels sprouts), radish, carrot, beets, eggplant, spinach, cucumber, squash, melons, cantaloupe, sunflowers and various ornamentals. Woody species include poplar, pine, sequoia, cedar, oak, etc.

[0085] The term "exogenous nucleic acid sequence" as used herein refers to a nucleic acid foreign to the recipient plant host or, native to the host if the native nucleic acid is substantially modified from its original form. For example, the term includes a nucleic acid originating in the host species, where such sequence is operably linked to a promoter that differs from the natural or wild-type promoter. In one embodiment, at least one nucleic acid sequence encoding *PFT1* or a variant thereof is operably linked with a promoter. It may be desirable to introduce more than one copy of an *PFT1* polynucleotide into a plant for enhanced expression. For example, multiple copies of the gene would have the effect of increasing production of the *PFT1* gene product in the plant.

[0086] Genetically modified plants of the present invention are produced by contacting a plant cell with a vector including at least one nucleic acid sequence encoding a *PFT1* or a variant thereof. To be effective once introduced into plant cells, the *PFT1* nucleic acid sequence is operably associated with a promoter which is effective in the plant cell to cause transcription of *PFT1*. Additionally, a polyadenylation sequence or transcription control sequence, also recognized in plant cells may also be employed. It is preferred that the vector harboring the nucleic acid sequence to be inserted also contain one or more selectable marker genes so that the transformed cells can be selected from non-transformed cells in culture, as described herein.

[0087] The term "operably linked" refers to functional linkage between a promoter sequence and a nucleic acid sequence regulated by the promoter. The operably linked promoter controls the expression of the nucleic acid sequence.

[0088] The expression of structural genes may be driven by a number of promoters. Although the endogenous, or native promoter of a structural gene of interest may be utilized for transcriptional regulation of the gene, preferably, the promoter is a foreign regulatory sequence. For plant expression vectors, suitable viral promoters include the 35S RNA and 19S RNA promoters of CaMV (Brisson, et al. *Nature*, 310, 511, 1984; and Odell, et al. *Nature*, 313, 810, 1985); the full-length transcript promoter from Figwort Mosaic Virus (FMV) (Gowda, et al. *J. Cell Biochem.* 13D, 301, 1989) and the coat protein promoter to TMV (Takamatsu, et al. *EMBO J.* 6, 307, 1987). Alternatively, plant promoters such as the light-inducible promoter from the small subunit of ribulose bis-phosphate carboxylase (ssRUBISCO) (Coruzzi, et al. *EMBO J.* 3, 1671, 1984; and Broglie, et al., *Science* 224, 838, 1984); mannopine synthase promoter (Velten, et al. *EMBO J.* 3, 2723, 1984) nopaline synthase (NOS) and octopine synthase (OCS)

promoters (carried on tumor-inducing plasmids of Agrobacterium tumefaciens) or heat shock promoters, e.g., soybean hsp17.5-E or hsp17.3-B (Gurley, et al. *Mol. Cell. Biol.* 6, 559, 1986; and Severin, et al. *Plant Mol. Biol.* 15, 827, 1990) may be used.

[0089] Promoters useful in the invention include both natural constitutive and inducible promoters as well as engineered promoters. The CaMV promoters are examples of constitutive promoters. To be most useful, an inducible promoter should 1) provide low expression in the absence of the inducer; 2) provide high expression in the presence of the inducer; 3) use an induction scheme that does not interfere with the normal physiology of the plant; and 4) have no effect on the expression of other genes. Examples of inducible promoters useful in plants include those induced by chemical means, such as the yeast metallothionein promoter which is activated by copper ions (Mett, et al. *Proc. Natl. Acad. Sci., U.S.A.* 90, 4567, 1993); In2-1 and In2-2 regulator sequences which are activated by substituted benzenesulfonamides, e.g., herbicide safeners (Hershey, et al. *Plant Mol. Biol.* 17, 679, 1991); and the GRE regulatory sequences which are induced by glucocorticoids (Schena, et al. *Proc. Natl. Acad. Sci., U.S.A.* 88, 10421, 1991). Other promoters, both constitutive and inducible will be known to those of skill in the art.

[0090] The particular promoter selected should be capable of causing sufficient expression to result in the production of an effective amount of structural gene product to modulate a photosensitive trait such as flowering. The promoters used in the vector constructs of the present invention may be modified, if desired, to affect their control characteristics.

[0091] Tissue specific promoters may also be utilized in the present invention. An example of a tissue specific promoter is the promoter active in shoot meristems (Atanassova, et al. *Plant J.* 2, 291, 1992). Other tissue specific promoters useful in transgenic plants, including the cdc2a promoter and cyc07 promoter, will be known to those of skill in the art. (See for example, Ito, et al. *Plant Mol. Biol.* 24, 863, 1994; Martinez, et al. *Proc. Natl. Acad. Sci. USA* 89, 7360, 1992; Medford, et al. *Plant Cell* 3, 359, 1991; Terada, et al. *Plant Journal* 3, 241, 1993; Wissenbach, et al. *Plant Journal* 4, 411, 1993).

[0092] Optionally, a selectable marker may be associated with the nucleic acid sequence to be inserted. As used herein, the term "marker" refers to a gene encoding a trait or a phenotype which permits the selection of, or the screening for, a plant or plant cell containing the marker. Preferably, the marker gene is an antibiotic resistance gene

whereby the appropriate antibiotic can be used to select for transformed cells from among cells that are not transformed. Examples of suitable selectable markers include adenosine deaminase, dihydrofolate reductase, hygromycin-B-phospho-transferase, thymidine kinase, xanthine-guanine phospho-ribosyltransferase and amino-glycoside 3'-O-phospho-transferase II (kanamycin, neomycin and G418 resistance). Other suitable markers will be known to those of skill in the art.

[0093] Vector(s) employed in the present invention for transformation of a plant cell include a nucleic acid sequence encoding *PFT1*, operably linked to a promoter. To commence a transformation process in accordance with the present invention, it is first necessary to construct a suitable vector and properly introduce it into the plant cell. Details of the construction of vectors utilized herein are known to those skilled in the art of plant genetic engineering.

[0094] *PFT1* nucleic acid sequences utilized in the present invention can be introduced into plant cells using Ti plasmids of Agrobacterium tumefaciens, root-inducing (Ri) plasmids, and plant virus vectors. (For reviews of such techniques see, for example, Weissbach & Weissbach, *Methods for Plant Molecular Biology*, Academic Press, NY, Section VIII, pp. 421-463, 1988; and Grierson & Corey, *Plant Molecular Biology*, 2d Ed., Blackie, London, Ch. 7-9, 1998; and Horsch, et al. *Science*, 227, 1229, 1985, both incorporated herein by reference). In addition to plant transformation vectors derived from the Ti or root-inducing (Ri) plasmids of Agrobacterium, alternative methods may involve, for example, the use of liposomes, electroporation, chemicals that increase free DNA uptake, transformation using viruses or pollen and the use of microprojection.

[0095] One of skill in the art will be able to select an appropriate vector for introducing the *PFT1*-encoding nucleic acid sequence in a relatively intact state. Thus, any vector which will produce a plant carrying the introduced DNA sequence should be sufficient. Even use of a naked piece of DNA would be expected to confer the properties of this invention, though at low efficiency. The selection of the vector, or whether to use a vector, is typically guided by the method of transformation selected.

[0096] The transformation of plants in accordance with the invention may be carried out in essentially any of the various ways known to those skilled in the art of plant molecular biology. (See, for example, *Methods of Enzymology*, Vol. 153, 1987, Wu and Grossman, Eds., Academic Press, incorporated herein by reference). As used herein, the

term "transformation" means alteration of the genotype of a host plant by the introduction of an *PFT1* or *PFT1* mutant nucleic acid sequence.

[0097] For example, an *PFT1* nucleic acid sequence can be introduced into a plant cell utilizing *Agrobacterium tumefaciens* containing the Ti plasmid, as mentioned briefly above. In using an *A. tumefaciens* culture as a transformation vehicle, it is most advantageous to use a non-oncogenic strain of *Agrobacterium* as the vector carrier so that normal non-oncogenic differentiation of the transformed tissues is possible. It is also preferred that the *Agrobacterium* harbor a binary Ti plasmid system. Such a binary system comprises 1) a first Ti plasmid having a virulence region essential for the introduction of transfer DNA (T-DNA) into plants, and 2) a chimeric plasmid. The latter contains at least one border region of the T-DNA region of a wild-type Ti plasmid flanking the nucleic acid to be transferred. Binary Ti plasmid systems have been shown effective to transform plant cells (De Framond, *Biotechnology*, 1, 262, 1983; Hoekema, et al., *Nature*, 303, 179, 1983). Such a binary system is preferred because it does not require integration into the Ti plasmid of *Agrobacterium*, which is an older methodology.

[0098] Methods involving the use of *Agrobacterium* in transformation according to the present invention include, but are not limited to: 1) co-cultivation of *Agrobacterium* with cultured isolated protoplasts; 2) transformation of plant cells or tissues with Agrobacterium; or 3) transformation of seeds, apices or meristems with Agrobacterium.

[0099] In addition, gene transfer can be accomplished by in planta transformation by Agrobacterium, as described by Bechtold, et al., (C. R. Acad. Sci. Paris, 316:1194, 1993). This approach is based on the vacuum infiltration of a suspension of *Agrobacterium* cells.

[0100] One method of introducing *PFT1*-encoding nucleic acid into plant cells is to infect such plant cells, an explant, a meristem or a seed, with transformed *Agrobacterium tumefaciens* as described above. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots, roots, and develop further into plants.

[0101] Alternatively, *PFT1* encoding nucleic acid sequences can be introduced into a plant cell using mechanical or chemical means. For example, the nucleic acid can be mechanically transferred into the plant cell by microinjection using a micropipette. Alternatively, the nucleic acid may be transferred into the plant cell by using polyethylene

glycol which forms a precipitation complex with genetic material that is taken up by the cell.

[0102] *PFT1* nucleic acid sequences can also be introduced into plant cells by electroporation (Fromm, et al., Proc. Natl. Acad. Sci., U.S.A., 82:5824, 1985, which is incorporated herein by reference). In this technique, plant protoplasts are electroporated in the presence of vectors or nucleic acids containing the relevant nucleic acid sequences. Electrical impulses of high field strength reversibly permeabilize membranes allowing the introduction of nucleic acids. Electroporated plant protoplasts reform the cell wall, divide and form a plant callus. Selection of the transformed plant cells with the transformed gene can be accomplished using phenotypic markers as described herein.

[0103] Another method for introducing *PFT1* nucleic acid into a plant cell is high velocity ballistic penetration by small particles with the nucleic acid to be introduced contained either within the matrix of such particles, or on the surface thereof (Klein, et al., Nature 327:70, 1987). Bombardment transformation methods are also described in Sanford, et al. (Techniques 3:3-16, 1991) and Klein, et al. (Bio/Techniques 10:286, 1992). Although, typically only a single introduction of a new nucleic acid sequence is required, this method particularly provides for multiple introductions.

[0104] Cauliflower mosaic virus (CaMV) may also be used as a vector for introducing nucleic acid into plant cells (U.S. Pat. No. 4,407,956). CaMV viral DNA genome is inserted into a parent bacterial plasmid creating a recombinant DNA molecule which can be propagated in bacteria. After cloning, the recombinant plasmid again may be cloned and further modified by introduction of the desired nucleic acid sequence. The modified viral portion of the recombinant plasmid is then excised from the parent bacterial plasmid, and used to inoculate the plant cells or plants.

[0105] As used herein, the term "contacting" refers to any means of introducing *PFT1* into the plant cell, including chemical and physical means as described above. Preferably, contacting refers to introducing the nucleic acid or vector into plant cells (including an explant, a meristem or a seed), via *Agrobacterium tumefaciens* transformed with the *PFT1* encoding nucleic acid as described above.

[0106] Normally, a plant cell is regenerated to obtain a whole plant from the transformation process. The immediate product of the transformation is referred to as a "transgenote". The term "growing" or "regeneration" as used herein means growing a

whole plant from a plant cell, a group of plant cells, a plant part (including seeds), or a plant piece (e.g., from a protoplast, callus, or tissue part).

[0107] Regeneration from protoplasts varies from species to species of plants, but generally a suspension of protoplasts is first made. In certain species, embryo formation can then be induced from the protoplast suspension, to the stage of ripening and germination as natural embryos. The culture media will generally contain various amino acids and hormones, necessary for growth and regeneration. Examples of hormones utilized include auxins and cytokinins. It is sometimes advantageous to add glutamic acid and proline to the medium, especially for plant species such as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these variables are controlled, regeneration is reproducible.

[0108] Regeneration also occurs from plant callus, explants, organs or parts. Transformation can be performed in the context of organ or plant part regeneration. (see Methods in Enzymology, Vol. 118 and Klee, et al., Annual Review of Plant Physiology, 38:467, 1987). Utilizing the leaf disk-transformation-regeneration method of Horsch, et al., Science, 227:1229, 1985, disks are cultured on selective media, followed by shoot formation in about 2-4 weeks. Shoots that develop are excised from calli and transplanted to appropriate root-inducing selective medium. Rooted plantlets are transplanted to soil as soon as possible after roots appear. The plantlets can be repotted as required, until reaching maturity.

[0109] In vegetatively propagated crops, the mature transgenic plants are propagated by utilizing cuttings or tissue culture techniques to produce multiple identical plants. Selection of desirable transgenotes is made and new varieties are obtained and propagated vegetatively for commercial use.

[0110] In seed propagated crops, the mature transgenic plants can be self crossed to produce a homozygous inbred plant. The resulting inbred plant produces seed containing the newly introduced foreign gene(s). These seeds can be grown to produce plants that would produce the selected phenotype, e.g. altered time to flowering.

[0111] Parts obtained from regenerated plant, such as flowers, seeds, leaves, branches, roots, fruit, and the like are included in the invention, provided that these parts comprise cells that have been transformed as described. Progeny and variants, and mutants of the regenerated plants are also included within the scope of the invention, provided that these parts comprise the introduced nucleic acid sequences.

[0112] Plants exhibiting a modulated photosensitive trait such as an altered time to flowering or an altered leaf number as compared with wild-type plants can be selected by visual observation. The invention includes plants produced by the method of the invention, as well as plant tissue and seeds. Stem elongation may be observed visually or preferably may be determined quantitatively by measuring with a ruler or with a video-imaging system.

[0113] In yet another embodiment, the invention provides a method for producing a genetically modified plant cell such that a plant produced from the cell has a modulated photosensitive trait such as an altered time to flowering compared with a wild-type plant. The method includes contacting the plant cell with an *PFT1* nucleic acid sequence to obtain a transformed plant cell; growing the transformed plant cell under plant forming conditions to obtain a plant having a modulated photosensitive trait such as an altered time to flowering. Conditions such as environmental and promoter inducing conditions vary from species to species, but should be the same within a species.

[0114] It will be understood by those of skill in the art that numerous and various modifications can be made without departing from the spirit of the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided herein for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLES

Example 1

Plant material and growth conditions.

[0115] Except when stated otherwise, all experiments were done in the Columbia accession. The alleles for the different mutants used were *phyA211* and *phyB9*. *FT* and *CO* overexpression lines were a gift of Detlef Weigel. Seeds were sterilized using chlorine in a vapor phase. For RNA extraction, total seedlings grown in MS media without sucrose or shoots from mature plants grown on soil were used. For hypocotyl measurements, seedlings were grown in water-agar medium. R and FR-light treatments were done in LED chambers (Percival Scientific) at 23 °C. WL sources were always from fluorescent tubes, 40-70 μmol/m^{2}s for LD and SD respectively, except for incandescent light extensions (LI) with 2 μmol/m^{2}s of PAR (400-700 nm).

[0116] For the genetic screen, T-DNA activation-tagged lines were obtained from the *Arabidopsis* Stock Center as pools representing around 100 lines each. Each pool was plated on water agar medium, and stratified for 3 days. Germination was induced by 1 h WL and then every 24 h seedlings received 5 min of R. At the 4th day seedlings were scored for signs of de-etiolation, such as shorter hypocotyls or partially opened cotyledons, and transplanted to soil to score adult phenotypes.

Example 2

Molecular characterization of *PFT1*

[0117] Genomic DNA was extracted from *pft1* mutants, cut with HindIII and EcoRI for right border rescue and with BamHI and SpeI for left border rescue, ligated and electroporated into SURE competent cells (Stratagene). Genomic DNA was successfully rescued with HindIII, BamHI and SpeI restriction enzymes. Specific oligonucleotides were designed to detect by PCR the T-DNA insertion in hemizygous and homozygous lines. The following 3 primers were used in PCR:

5'CAGAGGAACCCTGTTTCTACTGTTGAGCT3' (SEQ ID NO: 4),

5'CGTTACTTGGTTGAGCTTGGCCTGAAGGA3' (SEQ ID NO: 5) and

5'TCCCGGACATGAAGCCATTTATATGTA3' (SEQ ID NO: 6).

[0118] The expected PCR products were 563 bp for the WT and 491 bp for the mutant, while both bands were detected in hemizygotes.

[0119] The T-DNA co-segregated with the *pft1* mutation in 140 chromosomes analyzed. 36 F3 populations were tested and it was noted that the Basta resistance (conferred by the T-DNA) also co-segregated with the *pft1* mutation. Southern blot data and the rescued genomic DNA sequence agreed with a single insertion on BAC F2J7 from chromosome 1. The genomic sequence is shown in Figure 1 (SEQ ID NO: 1).

[0120] Intron-exon junctions were derived by comparison with EST clone APZL03h11R (Genbank accession# AV528220). RACE-PCR was performed using the GeneRacer kit according to the manufacturer instructions (Invitrogen)

[0121] The genomic *PFT1* clone was subcloned from BAC F2J7 in two consecutive steps into binary plasmid pPZP212, finally as a Pst1-SacI 8.5 kb fragment and introduced into *pft1* mutants by transformation with *Agrobacterium tumefaciens* (Clough, et al. *Plant J* 16, 735-43 (1998)). Transgenic lines were selected on MS media supplemented with 50 μg/ml of kanamycin, screened for single locus insertions in the T2 generation and the homozygous lines from the T3 generation used for physiological experiments.

[0122] To make the GFP fusion proteins, EcoRI sites were introduced in frame right before the ATG start codon (for GFP-*PFT1* fusions) and right before the TAA stop codon (for *PFT1*-GFP fusions). PCR amplified GFP coding region was subcloned into these EcoRI sites and final constructs confirmed by sequencing before being used for plant transformation as described above.

[0123] To assess a phenotype of overexpression, the *PFT1* cDNA (Figure 2; SEQ ID NO: 2) was subcloned in two steps as a BamHI-PstI-PstI fragment into CHF1 and CHF3 plasmids under the 35S constitutive promoter. Figure 2 shows the sequence of the cDNA. The predicted protein sequence is shown in Figure 3 (SEQ ID NO: 3). After plant transformation, lines were selected on kanamycin (CHF3 derived plasmids) or gentamycin (70 μg/ml CHF1 derived plasmids).

Example 3

mRNA quantitation

[0124] Specific mRNAs were extracted and quantified by RT-PCR, essentially as described (Blazquez, et al. *Plant Physiol.* 120, 1025-32 (1999)). mRNA was reverse transcribed using the 1st cDNA synthesis kit according to the manufacturer instructions (Invitrogen). Primers, annealing temperatures and the size of PCR products expected were as follows:

FT, 5'GCTACAACTGGAACAACCTTTGGCAAT 3' (SEQ ID NO: 7),
5'TATAGGCATCATCACCGTTCGTTACTC3'; (SEQ ID NO: 8) 63 °C, 365 bp;
CO, 5' AAACTCTTTCAGCTCCATGACCACTACT 3' (SEQ ID NO: 9),
5' CCATGGATGAAATGTATGCGTTATGGTTA 3', (SEQ ID NO: 10) 62 °C, 453 bp;
UBQ10, 5' GGTGTCAGAACTCTCCACCTCAAGAGTA 3'(SEQ ID NO: 11),
5' TCAATTCTCTCTACCGTGATCAAGATGCA 3' (SEQ ID NO: 12), 64°C, 318 bp.

[0125] In all cases at least one of the primers used for PCR spanned intron-exon junctions and amplification of genomic DNA was undetectable in non-retrotranscribed controls. PCR products were detected by southern blot using standard methodology and quantified using a phosporimager (Molecular Dynamics) in the exponential range of amplification.

Example 4

Phenotypes of *pft*1, *phy*A and *phy*B single, double and triple mutants

[0126] Consistent with a role in phytochrome signaling, *pft1* mutants displayed small but significant effects on hypocotyl length inhibition under both R and FR-light (Fig. 4a). *pft1* mutants were hypo-responsive to FR and hyper-responsive to R. These altered responses to light required functional *phyA* and *phyB*, suggesting that *PFT1* acts in both the phyA and phyB signaling pathways in seedlings.

[0127] *pft1* mutants displayed a late flowering phenotype when grown under long-day conditions (LD, 16 hr light/8 hr dark) (Fig. 4b). *phyA* and *phyB* regulate flowering time in opposing ways. *phyB* acts to delay flowering in both LD and short day (SD, 9hr light/16 hr dark), while phyA weakly promotes flowering, mainly in LD. Flowering time was measured under both LD and SD conditions in *pft1*, *pft1phyB*, and *pft1phyA* mutants, and the results were compared to *phyB* and *phyA* single mutants (Fig. 4b, c). *pft1* suppressed the early flowering time of suppressed the early flowering time of

phyB mutants in both LD and SD conditions independently of *phyA*, strongly suggesting the *PFT1* is essential for phyB regulation of flowering time. *pft1* suppression of *phyB* was specific for flowering, as the increased petiole length characteristic of *phyB* mutants was largely unaffected in the *pft1phyB* double mutant. Note that in *phyB* and *phyA phyB*, the apical meristem is already converted to an inflorescence meristem while no sign of change is observed in the *pft1* background. The effects of *phyA* and *pft1* mutations in delaying flowering under LD were not additive suggesting that *pft1* acts also downstream of phyA. This was further tested using extended photoperiods with low incandescent light (9 h WL/7 h LI/8 h D) where *phyA* is the main photoreceptor inducing flowering. Under these conditions, *phyA, pft1* and *phyApft1* double mutants showed a similar delay in flowering time, further supporting a role for *PFT1* in *phyA* signaling (total leaf number; WT: 9.67 ± 0.72; *phyA*: 52.5 ± 10.1; *pft1*: 43 ± 5.51; *phyApft1*: 56 ± 8.95; average ± SE of 9 plants).

Example 5

Molecular Characterization of *PFT1*

[0128] To characterize *PFT1* at the molecular level, the gene which had been tagged with a T-DNA insertion was cloned. The insertion produced a 29 bp deletion and localized within the transcription unit of a predicted gene. The gene structure was derived from the sequence of the EST clone APZL03h11R (Genbank # AV528220) and the 5' end was confirmed by RACE-PCR. It was concluded from this analysis that *PFT1* is a single copy gene with 15 exons (Fig. 5a). The predicted protein has 836 amino acids, a predicted vWFA (von Willebrand factor type A) domain in the N-terminus and a Gln rich region in the carboxy-terminus, reminiscent of some transcriptional activators (Fig. 5a). vWFA domains are widely distributed among all phyla (Ponting, et al. *J Mol Biol* 289, 729-45 (1999)). They are involved in various cellular processes and a high proportion have a divalent cation binding site that in some cases has been shown to mediate protein-protein interactions (Hinshelwood, et al. *J Mol Biol* 298, 135-47 (2000)). The DxSxS motif involved in coordination with a divalent cation is converted to ExSxA in *PFT1*. It is unclear whether this partially conserved motif can still bind a metal.

[0129] The expected *PFT1* mRNA is about 3 kb in length, consistent with the single band detected in northern blots. In *pft1* mutants, a lower molecular weight mRNA species was observed. RACE-PCR was used to further characterize this form and found that it corresponds to a 2.5 kb truncated form of *PFT1*, that initiates within the T-DNA.

This suggests that the 35S enhancers in the right border of the T-DNA initiate transcription within the gene. To confirm that the T-DNA insertion was the basis for the *pft1* phenotype, the seedling (Fig. 5b) and flowering time (Fig. 5c,d) phenotypes of *pft1* were rescued by transformation of a genomic copy of the predicted wild-type gene. Overexpression of *PFT1* caused an early flowering phenotype, suggesting that PFT is limiting for flowering (Fig. 5d). Transformation of *pft1* plants with *PFT1* cDNA under the strong 35S promoter also produced a high proportion of co-suppressed plants, whose phenotypes were not more severe than the *pft1* mutant. These data lend support to the interpretation that the *pft1* allele is a strong or null allele (Fig. 5d and data not shown). In a separate experiment, plants overexpressing *PFT1* were early flowering at lower temperatures indicating that *PFT1* may also be involved in responses to temperature (data not shown).

[0130] To study the subcellular localization of *PFT1*, *GFP* was fused to either the N or C-terminus of *PFT1* in the context of the genomic clone. These constructs encode a functional protein as they complemented the *pft1* phenotype (data not shown). Both N and C terminal GFP fusions localize to the nucleus (data not shown). The GFP fusions in plants grown under R, FR and WL (white light) were also analyzed. Differences in *PFT1* localization were not observed (data not shown) suggesting that *phy*B regulation of *PFT1* activity might occur postranscriptionally or through association with other proteins.

Example 6

FT and *CO* mRNA levels in WT, *phy*B, *pft1* and *pft1 phy*B mutants

[0131] To test the nature of *phyB* suppression by *pft1*, the role of *pft1* in the regulation of key flowering time genes was investigated. *FLOWERING LOCUS T (FT)* is an integrator of several flowering time pathways, including the photoperiod pathway. *FT* expression has been shown to be lower in *phyA* and *cry2* mutants in conditions where these mutants are late flowering (Yanovsky et al. *Nature* 419, 308-312 (2002)). The expression patterns of *FT* mRNA in WT, *phyB*, *pft1* and *phyBpft1* double mutants were analyzed. Expression levels in 8-day old seedlings were first tested, as previously described (Blazquez, et al. *Plant Physiol* 120, 1025-32 (1999)), at the first day where plants grown in LD are committed to flowering (Bradley, et al. *Science* 275, 80-3 (1997)). In LD-grown seedlings, *FT* expression was significantly higher in *phyB* mutants compared to WT (Fig. 6b), suggesting that *phy*A and *phy*B have opposing roles in

flowering by differentially regulating *FT* expression. In contrast, these differences were not found in 8 day-old seedlings grown in SD (data not shown). This could be due to the lack of commitment of SD grown seedlings at this early stage of development (note that WT plants bolt at 70 days in these conditions). Thus, *FT* mRNA levels in 26-day old plants grown in SD were tested. At this time point, *phyB* mutants are approximately two weeks from flowering, a similar difference between the commitment to flower and the actual bolting time when grown in LD (Fig 4b, c and data not shown). Under these conditions, a large increase in *FT* mRNA levels in *phyB* mutants compared to WT plants (Fig. 6a-b) was observed. These data thus provide a molecular mechanism for the early flowering time of *phyB* mutants in both LD and SD. Moreover, *FT* mRNA levels were low in *pft1* and *phyBpft1* double mutants in all the conditions tested (Fig. 6a-b), suggesting that this is the molecular mechanism for the suppression of the early flowering time phenotype of *phyB* mutants by *pft1*.

[0132] CONSTANTS (*CO*) is a key component of the photoperiod pathway which integrates information from the circadian clock (Suarez-Lopez, et al. *Nature* 410, 1116-20 (2001)) as well as phyA and cry2 signaling (Yanovsky, et al. *Nature* 419, 308-12 (2002)). CO also directly activates *FT* expression (Samach, et al. *Science* 288, 1613-6 (2000)). Higher levels of *CO* mRNA in LD grown *phyB* seedlings were found compared to WT and lower levels were found in the *pft1* background, thus suggesting that *PFT1* could function upstream of CO in the regulation of *FT* (Fig. 6a). However in SD grown plants, increased levels of *CO* in *phyB* mutants were not detected, nor were the levels decreased in the *pft1* background (Fig. 6c). This is consistent with a role for CO specific to LD grown plants. These data suggest that if *phy*B regulates flowering through *PFT1* by lowering *CO* mRNA levels, this is not the only mechanism. A different pathway must exist, especially in SD. *PFT1* could activate *FT* expression by regulating *CO* activity or simply act in a parallel pathway, independently of *CO*. Consistent with this latter possibility, loss of *CO* only partially suppresses the early flowering time of *phyB* mutants. Both *FT* and *CO* overexpression produce a strong early flowering phenotype that is independent of the presence of *PFT1* (Fig. 7a, b) supporting a model in which *FT* is activated downstream of *PFT1* while *CO* may act downstream, but also act in a parallel pathway to *PFT1* (Fig. 7c).

[0133] The data presented so far suggests that *PFT1* is a positive regulator of flowering whose action is inhibited by *phyB*. *PFT1* mRNA levels are slightly elevated in

phyB mutants relative to WT (Fig. 8). This increased expression cannot fully explain the effects of *PFT1* on flowering time, however, as *PFT1* overexpression lines did not flower as early as *phyB* mutants (compare Fig. 4c and 5d). These results suggest that regulation of *PFT1* activity by *phyB* is subject to more complex mechanisms.

[0134] The *phyB* mutation has a stronger inductive effect in non-inductive photoperiods (SD in long-day plants and LD in short-day plants) suggesting a role in photoperiod sensing. However, the accelerated flowering of *phyB* mutants could be the result of a general inhibitory effect that is independent of daylength. Consistent with these observations a "light quality" pathway has been proposed (Simpson, et al. *Science* 296, 285-9 (2002)) to explain the role of *phyB* and other stable phytochromes (*phy*D and *phy*E in *Arabidopsis* in flowering. This light quality perception pathway may be involved in the shade avoidance syndrome triggered in response to lower red/far-red ratios (Ballare, C.L. *Trends Plant Sci* 4, 201 (1999)). In contrast to *co* and *cophyB* double mutants, *pft1* and *pft1phyB* double mutants show a strong response to photoperiod (Fig. 4b, c). This observation strongly suggests an important role of *phyB* and *PFT1* in a photoperiod-independent pathway, likely, a "light quality pathway". In support of this view, FR pulses following each SD period greatly accelerated flowering in WT plants, but not in *pft1* mutants (total leaf number; WT:16 ± 3.29; *pft1*: 39.9 ± 2.9) compared to SD grown plants (Fig 4c). A model is proposed where PFT1 acts in this "light quality pathway" downstream of *phy*B. The resulting signal is then integrated with the photoperiod pathway through the modulation of *FT* transcription, which may involve the action of *CO*.

Example 7

Expression of the *PFT1* genes in tobacco

[0135] The *PFT1* gene is transformed into tobacco plants. To confirm that expression of *PFT1* in other plants leads to the *PFT1* phenotype the *PFT1* gene is transformed and expressed in tobacco using the method described by Gallois et al. (Methods Mol. Biol (1995) 49:39). The transgenic tobacco plants have phenotypes identical or similar to the phenotypes observed in *Arabidopsis*.

Example 8

Expression of *PFT1* genes in rice

[0136] The *PFT1* gene as represented by SEQ ID NO:1 or by SEQ ID NO 16 is introduced into rice embryos employing a commercially available ballistic micro-projectile device. The bombarded embryos are grown on N6 medium (see Chu et al.,

Scientia Sinica, 18:659-668, 1975) containing hygromycin. Regenerated hygromycin resistant plants are analyzed by PCR and Southern blot analysis for the presence of inheritable *PFT1* DNA. The results indicate that the genomic DNA of the rice transformants contain inheritable copies of the transgene.

Example 9

Expression of *PFT1* genes in tomato

[0137] The transformation of seedlings of L. esculentum cv. UC82 (grown from seeds obtained from Ferry Morse Seed Co., Modesto, Calif.) is done according to the protocol of Fillatti et al. [(1987) Bio/Technology 5:726-730], with modifications as described below.

[0138] Cotyledons are excised from eight-day-old tomato seedlings germinated in vitro and cut into three sections. The middle sections with dimensions 0.5 cm x 0.25 cm are placed abaxile side up on one-day-preconditioned tobacco feeder plates containing KCMS incubation medium [Murashige and Skoog salt base (Gibco Laboratories, Grand Island, N.Y.) with thiamine-HCl, 1.3 mg/l; 2,4-dichlorophenoxyacetic acid, 0.2 mg/l; kinetin, 0.1 mg/l; monobasic potassium phosphate, 200 mg/l; myo-inositol, 100 mg/l; sucrose, 30 mg/l; tissue culture agar, 8 g/l, pH 5.7] and are incubated at 27 °C with 16 hours of light per day.

[0139] The tobacco feeder plates are prepared according to the method of Horsch and Jones [(1980) In Vitro 16:103-1089] with the following modifications. Cells from a six-day-old tobacco suspension culture are resuspended in fresh MM medium (Murashige and Skoog salt base with thiamine-HCl, 0.1 mg/l; pyridoxine-HCl, 0.5 mg/l; nicotinic acid, 0.5 mg/l; glycine, 2 mg/l; 6-benzylaminopurine, 0.5 mg/l; 2,4-dichlorophenoxyacetic acid, 0.5 mg/l; myo-inositol, 100 mg/l; sucrose, 30 g/l, pH 5.7) to a final density of 0.3 g fresh weight per ml. The suspension were stirred, and 1.5 ml aliquots are pipetted onto KCMS medium (25 ml) solidified with tissue culture agar (0.8% w/v) in 100 mm x 20 mm plastic petri plates.

[0140] After one day of incubation of tomato cotyledon tissue on the tobacco feeder plates, the explants were floated in 20 ml of Murashige and Skoog liquid medium without hormones containing an overnight culture of *Agrobacterium tumefaciens* strain LBA4404 [Clontech, Palo Alto, Calif.; see also Ooms et al. (1982) Plasmid 7:15-19],

harboring a transformation vector containing *PFT1*, which were inserted into *A. tumefaciens* through triparental mating.

[0141] *A. tumefaciens* strain LBA4404 is maintained at a density of 5 x 10^8 cells/ml. The tissue and *Agrobacterium* are incubated at room temperature for 30 min, after which time the explants are blotted on sterile Whatman paper No. 1 and transferred to tobacco feeder plates. The cultures are incubated at 27 °C with 16 hr of light per day. After two days of incubation, the treated cotyledon segments are transferred to regeneration 2Z medium (Murashige and Skoog salt base with thiamine-HCl, 1.0 mg/l; pyridoxine-HCl, 0.5 mg/l; nicotinic acid, 0.5 mg/l; glycine, 2 mg/l; zeatin, 2 mg/l; sucrose, 30 g/l; myo-inositol, 100 mg/l; tissue culture agar, 8 g/l, pH 5.7) with 500 μg/ml cefotaxime and 100 μg/ml kanamycin).

[0142] The cultures are being incubated at 27 °C with 16 hours of light per day under 4,000 lux of light intensity. When kanamycin-resistant shoots reach a height of one inch, they will be rooted on rooting medium, which is identical to regeneration 2Z medium except that it lacks hormones and contains 250 μg/ml cefotaxime and 50 μg/ml kanamycin. The transgenic shoots will then be grown into fruit-bearing transgenic tomato plants.